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Retinal Microvascular Responses to Short-Term Changes in Particulate Air Pollution in Healthy Adults

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Abbreviations

BC: Black Carbon

BMI: Body Mass Index

CRAE: Central Retinal Arteriolar Equivalent

CRVE: Central Retinal Venular Equivalent

DBP: Diastolic Blood Pressure

IQR: Interquartile Range

PP: Pulse Pressure

PM_{10}: Particulate Matter with a diameter smaller than 10 μm

$PM_{2.5}$: Particulate Matter with a diameter smaller than 2.5 μm

MESA: Multi-Ethnic Study of Atherosclerosis

eNOS: endothelial Nitric Oxide Synthase

NO: Nitric Oxide

SBP: Systolic Blood Pressure

Abstract

Background: The microcirculation plays an important role in the physiology of cardiovascular health. Air pollution is an independent risk factor for the development and progression of cardiovascular diseases, but the number of studies on the relation between air pollution and the microcirculation is limited.

Objectives: To examine the relationship between short-term changes in air pollution and microvascular changes.

Methods: We measured retinal microvasculature using fundus image analysis in a panel of 84 healthy adults (52% women) aged 22 to 63y between January and May 2012. Blood vessels were measured as Central Retinal Arteriolar/Venular Equivalent (CRAE/CRVE). The median number of measurements was 2 (range: 1-3). We used monitoring data on particulate air pollution (PM_{10}) and black carbon (BC). Mixed-effect models were used to estimate associations between CRAE/CRVE and exposure to PM_{10} and BC using various exposure windows.

Results: CRAE and CRVE were associated with PM_{10} and BC concentrations, averaged over 24 hours before the retinal examinations. Each 10-µg/m³ increase in PM_{10} was associated with a 0.93 µm decrease (95% CI: -1.42, -0.45; p=0.0003) in CRAE, and a 0.86-µm decrease (95% CI: -1.42, -0.30; p=0.004) in CRVE after adjustment for individual characteristics and time varying conditions such as ambient temperature. Each 1-µg/m³ increase in BC was associated with a 1.84 µm decrease (95% CI: -3.18, -0.51; p<0.001) in CRAE.

Conclusions: These findings suggest that the retinal microvasculature responds to short-term changes in air pollution levels. These results support a mechanistic pathway through which air pollution can act as a trigger of cardiovascular events at least in part through effects on the microvasculature.

Introduction

Exposure to ambient levels of air pollution increases the incidence of cardiovascular mortality and morbidity (Nawrot et al. 2007; Zanobetti et al. 2003). Research indicates that different fractions of particulate air pollution contribute to the development of cardiovascular disease and provoke cardiovascular events (Brook et al. 2010; Dockery et al. 1993; Nawrot et al. 2011). PM_{10} (particles less than 10 μm in diameter) is a complex mixture of compounds including transition metals, sulfate and nitrate salts and black carbon (Wilson and Suh 1997). Black carbon (BC) is a measure of traffic-related particles that are produced as a combustion by-product.

Although the microcirculation makes up the bulk of the circulatory system, its role in cardiovascular disease remains less clear than the influence of the macrocirculation (Liew et al. 2008). There are two main theories about the significance of microvascular changes in the context of cardiovascular disease. First, microvascular changes could be an early marker for cardiovascular disease, secondary to the disease process (Wong et al. 2004a). Alternatively, microvascular changes could be a primary cause for the development of cardiovascular changes (Levy et al. 2001; Mulvany 1991; Wang et al. 2008). Central Retinal Arteriolar Equivalent is a predictor of future hypertension (Wang et al. 2008). Recent evidence suggests an association between air pollution exposures and markers of microvascular effects (Adar et al. 2010; Barath et al. 2010; Tornqvist et al. 2007).

Changes in the microcirculation can be explored non-invasively by studying retinal blood vessels that are visualized in fundus images (Wong et al. 2001; Wong and Mitchell 2007). The retinal blood vessels have anatomical and physiological features that are comparable with the coronary circulation. Pathologies of the retinal blood vessels parallel changes in the coronary micro- and

macrocirculation (Nguyen and Wong 2006; Tedeschi-Reiner et al. 2005; Tso and Jampol 1982). Retinal vessel caliber is an independent predictor for cardiovascular diseases, with arterial narrowing acting as a marker for arteriolar damage and predicting hypertension, and venular widening has been associated with inflammation, endothelial dysfunction, and markers of atherosclerosis (Nguyen and Wong 2006; Wong et al. 2004a; Wong and Mitchell 2007).

Adar and coworkers (2010) were the first to associate exposure to air pollution with arteriolar narrowing. Among 4,607 participants of the Multi-Ethnic Study of Atherosclerosis (MESA), Central Retinal Arteriolar Equivalent (CRAE) narrowed by 0.8 μm (95% CI: -1.1, -0.5) in association with an interquartile increase in long-term exposure (3 $\mu g/m^3$ $PM_{2.5}$ during the 2 years preceding the clinical exam). The magnitude of this change corresponded to the change in CRAE associated with a 7-year increase in age in their study population. In a cross-sectional analysis investigating exposure on the previous day, CRAE narrowed by 0.4 μm (95% CI: -0.8, -0.04) in association with a 9-$\mu g/m^3$ increase in $PM_{2.5}$ (Adar et al. 2010).

Here, we report on a study of short-term air pollution exposures and microvascular changes in healthy adults (age 22 – 63 years) using a repeated measures design.

Methods

Study population

The study was conducted in Belgium between January 2012 and May 2012 and included employees of the Flemish Institute for Technological Research (VITO). A total of 183 persons were contacted and 84 (46%) agreed to participate in the study. Participants were 22 to 63 years

old. All VITO employees undergo an annual clinical examination and all study participants were free of clinical cardiovascular diseases and diabetes before and during the study period.

Participants were not asked to fast before study visits and their post-prandial status was not recorded. On each study day, participants completed a questionnaire on their current medical history and smoking status, as well as on the use of alcohol, coffee and specific medications, and time spent in traffic during the 24 hours prior to the clinical visit. 84 persons participated in our study, of which 32 (38%) completed one visit, 7 (8%) completed two visits, and 45 (54%) participated in all three clinical visits. The visits were scheduled between 9 am and 5 pm and took place on the campus of the Flemish Institute for Technological Research. The visits were on average 16 days apart (range: 14 to 18 days). The clinical visits were scheduled on the same time of day [mean difference 1.5 hour (range: 0.2 to 2.2 hours)]. Participants gave their written informed consent. The Ethics Board of Hasselt University and University Hospital Antwerp approved the study.

Retinal photography and grading

The fundus of the right eye of each participant was photographed using a Canon 45° 6.3 megapixel digital non-mydriatic retinal camera (Hospithera, Brussels, Belgium). Participant characteristics were masked for the trained grader before review and analysis of the retinal images. IVAN retinal image analysis software was used to measure retinal vessel diameters according to previously reported protocols (Hubbard et al. 1999; Knudtson et al. 2003; Wong et al. 2004b). Diameters were summarized as the Central Retinal Arteriolar Equivalent (CRAE) and Central Retinal Venular Equivalent (CRVE). The equivalents represent a summary of vessel diameters within an area equal to 0.5-1 disc diameters from the optic disc margin.

Cardiovascular parameters

Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate were measured with an automated device (Stabilograph, Stolberg, Germany), according to the guidelines of the European Society of Hypertension (Parati et al. 2008). After the subjects had rested for 5 min, the blood pressure and heart rate were measured five times consecutively. The average of the last three measurements was calculated and used in data analyses. These cardiovascular parameters were only measured during the second and third clinical examination (n = 59).

Outdoor temperature and barometric pressure

The 24-hour mean outdoor temperature and barometric pressure measured at the nearby Retie meteorological station (N° 06464; 51°13'50.29" N, 5°3'7.64" E) were obtained from the Belgian Royal Meteorological Institute.

Air pollution levels: exposure assignment

Ambient air pollution levels were measured at a nearby official monitoring station in Dessel (N° 42N016; 51°14'2.92" N, 5° 9'45.58" E) and the data were obtained from the Flemish Environmental Agency. The distance from the monitoring station to the campus of the Flemish Institute for Technological Research is between 5.4 and 9.5 km. The station monitors ambient concentrations of a range of air pollutants, including PM_{10} and black carbon, every 30 min. PM_{10} was measured with beta-absorption, whereas black carbon was measured using reflectometry and transmission techniques.

For each participant, average air pollution concentrations were determined for the 2, 4, 6, and 24 hours before the retinal exam (lag 2h, 4h, 6h, and 24h, respectively). Air pollution levels were

also assigned as a 24-hour average for the previous calendar day (lag 1d) and 48-hour average for the two calendar days preceding the retinal exam (lag 2d).

Statistical analysis

We performed pollutant-specific exposure-response analyses using mixed models that included random effects for each participant across the clinical examinations (SAS version 9.2, SAS Institute Inc, Cary, NC). This method allows each subject to serve as his or her own control over time and eliminates within-subject confounding by personal characteristics that do not change over time. Associations with exposures over different lag periods (lag 2h to lag 2d) were estimated in separate models. We did descriptive analyses to identify potential predictors of the markers of the microcirculation that could modify or confound the association between the microcirculation and air pollution exposure. All analyses were adjusted for gender, age, body mass index (BMI), smoking status, alcohol and coffee consumption during the 24 hours prior to the examination, day of the week, time of day, outdoor temperature, and barometric pressure.

In a series of sensitivity analyses, we also adjusted for blood pressure (SBP, DBP) and heart rate in a subset of 59 participants, and adjusted for fellow vessel diameter (i.e., for CRVE in models of CRAE, and vice versa). In addition, we repeated analyses with smokers (n=3) and individuals currently using medication (n=2) excluded. To explore the shape of the dose-response curves we estimated associations between average PM_{10}-concentrations over different lags and the microcirculation markers estimated using unadjusted models with exposures modeled as restricted cubic splines with 5 knots at the 5th, 25th, 50th, 75th and 95th percentiles (Harrell 2001). Finally, differences in between- and within-subject air pollution effects could be possible.

Therefore, we fitted separate mixed models that included terms for within- and between- subject exposure effects in addition to the overall model. All tests were two-sided.

Results

Characteristics of the study population are summarized in Table 1. 52% of included participants are women. The population had a mean age of 37 ± 9 years. All participants reported that they were free of diabetes and cardiovascular disease, though one used medication for blood pressure control (an angiotensin receptor blocker) and one used cholesterol lowering medication (a statin). Three participants were active smokers. All participants had a university or college degree. Short-term air pollution concentrations were highly variable during the study. PM_{10} concentrations (lag 24h) ranged from 9.7 to 117.7 μg/m³, with interquartile ranges (IQR) of 9.6, 39.1, and 3.7 μg/m³ for the first, second, and third visits, respectively. BC concentrations ranged from 0.37 to 6.99 μg/m³, with IQRs of 0.94, 5.64, and 0.29 μg/m³ for the first, second, and third visits. During the 5-month study period, the daily outdoor temperature ranged from -6.8 to 20.2 °C and the barometric pressure from 993 to 1031 hPa. No within-person correlation was observed for the different exposure periods. Seventy-four participants reported that they spent on average 84 min (± 20) in traffic driving a car during the previous 24 hours. Of these 74 participants, 24 participants reported driving an average of 8 min (± 22) in congested traffic. Twenty-seven participants reported riding a bicycle in traffic (mean duration 9 min ± 20).

Predictors and correlates of CRAE and CRVE

Central Retinal Arteriolar Equivalent (CRAE) and Central Retinal Venular Equivalent (CRVE) averaged 136 μm (± 14 μm) and 189 μm (± 18 μm), respectively. The CRAE/CRVE ratio was 0.722 (± 0.067). CRAE did not differ significantly between men and women (p=0.95), but

decreased by 0.59 µm (95% CI: -0.94, -0.23; p=0.0015) in association with a 1-year increase in age. BMI (p=0.97), alcohol use (p=0.58), coffee consumption (p=0.28), outdoor temperature (p=0.58), and barometric pressure (p=0.97) were not significant predictors of CRAE, nor was time of day (p=0.34). A 10-min increase in the amount of time spent in driving a car was associated with a 0.14 µm decrease (95% CI: -0.35, 0.07; p=0.18) in CRAE. Finally, a 1-µm increase in CRVE was associated with a 0.40 µm increase in CRAE (95% CI: 0.30, 0.51; p<0.0001). Outdoor temperature was the only statistically significant predictor of CRVE (0.98 µm decrease with a 1-°C increase in outdoor temperature, 95% CI: -1.33, -0.45; p=0.0001).

Microcirculatory markers in association with changes in short-term air pollution

Unadjusted models of associations between CRAE and PM_{10} modeled using restricted cubic splines did not indicate a threshold effect (Figure 1). An increase in PM_{10} within the low concentration ranges (<30 µg/m³) was associated with a decrease in CRAE for lag 1d and lag 2d. Studying the shape of the association showed no threshold effect at higher concentrations and a linear shape (at lag 24h from 30 µg/m³ onwards) over the full exposure range (Figure 1).

After adjustment for gender, age, BMI, smoking, alcohol and coffee consumption 24 hours prior to the examination, time of the day, day of the week, 24-hour mean outdoor temperature and barometric pressure, CRAE was associated inversely with the PM_{10} and BC concentration in the hours before and the days before the clinical examination (Table 2). Each 10-µg/m³ increase in average PM_{10} during the previous 24 hours was associated with a 0.93 µm decrease (95% CI: -1.42, -0.45; p=0.0003) in CRAE (Table 2, model 1). Significant negative associations were also estimated between CRAE and average PM_{10} over shorter exposure windows, and for PM_{10} averaged over the previous 2 days. A 1-µg/m³ increase in BC during the previous 24 hours also

was negatively associated with CRAE (-1.84 μm; 95% CI: -3.18, -0.51; p=0.008), but associations with shorter and longer exposure periods were not significant (Table 2, model 1). All associations with CRAE moved toward the null when adjusted for CRVE in addition to the other covariates (Table 2, model 2) but statistically significant negative associations persisted for 24h average exposures to both PM_{10} and BC.

CRVE was negatively associated with a 10-μg/m³ increase in PM_{10} during the previous 24 hours (-0.86 μm; 95% CI: -1.42, -0.30; p=0.004) and with PM_{10} exposure during other lag periods (Table 3, model 1). A 1-μg/m³ increase in BC during the previous 24h was also negatively associated with CRVE, though the association was not significant (-1.18 μm; 95% CI: -3.11, 0.75; p=0.23). Most associations moved closer to the null after adjustment for CRAE.

Sensitivity analyses

We did not find statistically significant associations between PM_{10} or BC and blood pressure components (systolic blood pressure (SBP), diastolic blood pressure (DBP), or pulse pressure (PP)) in the subset of 59 participants with blood pressure data (Supplemental Material, Table S1). When we adjusted for SBP, DBP, and heart rate, in addition to model 1 covariates and CRAE or CRVE, only the association between 24h PM_{10} and CRAE was significant (-0.50 μm; 95% CI: -0.92, -0.08; p=0.005) though a 1-μg/m³ increase in 24h BC was also negatively associated with CRAE (-1.08 μm; 95% CI: -2.21, 0.04; p=0.059) (Supplemental Material, Table S2). No significant associations between CRVE and air pollution indicators were estimated based on this model.

Associations between CRAE and 24h average PM_{10} and BC persisted when we also adjusted for time spent in traffic, and when we excluded the three smokers and two participants on anti-

hypertensive and/or cholesterol medication (data not shown). The negative associations with 24h PM_{10} and BC were also confirmed when we excluded the 32 participants with only one CRAE measurement (n=52) [estimated mean decreases of 0.76 μm (95% CI: -1.32, -0.20; p=0.01) and 1.37 μm (95% CI: -2.90, 0.15; p=0.07) for a 10-μg/m³ increase in 24h PM_{10} and a 1-μg/m³ increase in BC, respectively]. Associations were of approximately the same magnitude (though no longer significant) when data from the 2nd set of study visits, which took place during a time of relatively high PM_{10} and BC concentrations, were excluded (data not shown).

Finally, we ran models to differentiate between the within- and between-subject effects. Our overall estimates for PM_{10} were driven by the within-subject effects. Within-subject effect estimates indicated that each 10-μg/m³ increase in 24h PM_{10} was associated with a 0.66 μm decrease in mean CRAE (95% CI: -1.02, -0.30; p=0.0005) and each 1-μg/m³ increase in 24h BC was associated with a 1.08 μm decrease in CRAE (95% CI: -2.02, -0.13; p=0.03) (Supplemental Material, Table S3). Corresponding estimates for between-subject effects were -1.34 (95% CI: -2.82, 0.13; p=0.07) and -3.68 (95% CI: -6.33, -1.02; p=0.007), respectively.

Discussion

We found a decrease in CRAE or Central Retinal Arteriolar Equivalent in association with exposure to PM_{10} and BC in a panel of healthy adults. These results remained significant after adjustment for gender, age, BMI, systolic and diastolic blood pressure or any of the other covariates studied. Arteriolar narrowing is an independent predictor of risk of myocardial infarction, hypertension, and cardiovascular mortality (Cheung et al. 2007a; Cheung et al. 2007b; Wong et al. 2002; Wong et al. 2006b).

Other authors have reported an association between blood pressure and acute changes in air pollution (Hoffmann et al. 2012, Jacobs et al. 2012, Wu et al. 2013). Despite the decrease in retinal arteriolar vessel diameter, we did not observe statistically significant associations between PM_{10} or BC and blood pressure in the subset of participants with blood pressure data. We propose three explanations for this lack of association in our study. First, blood pressure is a highly variable phenotype, which is regulated by several control mechanisms counteracting changes in vessel diameter (Brook et al. 2002). This study might not have sufficient power to detect such an effect. Second, the small vasoconstriction in the retinal blood vessels might not change overall peripheral resistance, thus blood pressure levels remain normal. Third, microvascular changes can be a cause or a consequence of elevated blood pressure. In our healthy population, air pollution exposure was associated with microvascular changes after adjustment for blood pressure. The microvasculature might rather be a target for primary changes that might eventually result in elevated blood pressure rather than vice versa. This is in agreement with the hypothesis that microvascular changes can be a primary cause for the development of cardiovascular changes (Levy et al. 2001; Mulvany 1991; Wang et al. 2008). In another study, inhalation of air pollution was associated with acute vasoconstriction of the forearm conduit artery without changes in systemic blood pressure (Brook et al. 2002).

Both fellow vessel diameter and blood pressure components are known to influence the microvascular changes in the retina (Cheung et al. 2007a; Cheung et al. 2007b; Wong et al. 2002; Wong et al. 2006b). The effect estimates were attenuated by adjusting for fellow vessel diameter (i.e., including CRVE in models of associations between the exposures and CRAE, and vice versa) (Table 2 and Table 3, model 2), and much less by blood pressure (Supplemental

Material, Table S2). Additional research is needed to clarify the relation between the pollutants, blood pressure and CRAE or CRVE.

It is likely that both vessel diameters are affected by an identical mechanism and respond in the same way (Liew et al. 2007; Miller et al. 2012). Due to their proximity, these blood vessels could interact by exchanging biologically active agents (Kavdia and Popel 2006). A model that accounts for fellow vessel diameter represents the independent effects of air pollution on both vessels (CRAE/CRVE), but due to their correlation over-adjustment cannot be excluded.

Exposure to air pollution has been associated with markers of pulmonary inflammation, which can cause a low-grade, systemic inflammation (Chuang et al. 2007; Hoffmann et al. 2009). Inflammation has been linked with endothelial dysfunction (Stenvinkel 2001). The effects of the systemic inflammation reaction may take some time to affect the retinal blood vessels. We hypothesize that inflammatory responses may alter the activity of the endothelium and initiate endothelial dysfunction, which may result in the narrowing of the retinal arterioles even up to several hours after exposure. Given the high variation in ambient air pollution levels, with intermittent peak episodes, the microvasculature is constantly adapting to a changing environment. Our findings suggest that this might occur very fast, even within 24 hours. In our first model, exposure to PM_{10} during all the hourly exposure windows was inversely associated with CRAE.

To our knowledge, only Adar et al. (2010) have previously published a study of short-term effects of air pollution on the human retinal microvasculature. The microvascular changes reported in our study complement those found by Adar and coworkers, who reported changes in the retinal microcirculation associated with long-term exposure (averaged over the previous 2

years) and short-term exposure (averaged over the previous day) in a cross-sectional analysis using the MESA cohort. Assuming that 9 μg/m³ $PM_{2.5}$ corresponds to 12.9 μg/m^3 PM_{10} (Nawrot et al. 2011), the short-term cross-sectional association reported by Adar et al. (-0.4 μm; 95% CI: -0.8, -0.04) per 9μg/m³ increase in average $PM_{2.5}$ on the previous day, is smaller than our estimate based on repeated measurements (-1.20 μm; 95% CI: -1.61, -0.61). The effect size reported in our study may be larger than the one reported for the MESA cohort because our study population was exposed to greater variation in PM_{10} and BC concentrations. Furthermore, our study population consisted of young, healthy people with the same socio-economic status, in contrast with the much older and more diverse MESA cohort. In theory, arteriolar narrowing in response to air pollution in healthy people might be more pronounced than in susceptible people. A healthy microvasculature may respond better to changing conditions. This healthy response could result in bigger microvascular changes, whereas the response in susceptible people or people at risk might be compromised due to the already affected microvasculature.

Our results are consistent with previously reported health effects of air pollution. Toxicological studies have revealed that short-term exposure to peak levels of air pollutants is associated with microvascular responses. Animal studies conducted by Nurkiewicz et al. demonstrated that exposure to (ultrafine) particulate matter induced oxidative stress that led to eNOS-uncoupling and reduced bioavailability of the vasodilator NO (Nurkiewicz et al. 2004; Nurkiewicz et al. 2006; Nurkiewicz et al. 2011). In addition controlled exposure studies of humans have reported evidence of impaired macrovascular endothelial function in response to diesel exhaust (Barath et al. 2010; Tornqvist et al. 2007).

Existing evidence suggests that air pollution is able to trigger an acute autonomic imbalance, favoring sympathetic nerve activity to the smooth muscles surrounding blood vessels (Pieters et

al. 2012). Increased sympathetic activity causes smooth muscle contraction and thus vasoconstriction. Retinal blood vessels lack functional sympathetic innervations (Riva et al. 1986), therefore, autonomic imbalance is not likely to be the primal cause of retinal arteriolar vasoconstriction. This might also explain why microvascular changes were more pronounced for the 24 hours exposure window than for the shorter lags.

Previously reported experiments on forearm conduit arteries allow assessing endothelial function, but the retinal blood vessels share more similarities in development and anatomy with the microvasculature of the heart, lungs and the brain (Wong et al. 2006a). Therefore, changes in retinal blood vessels may be related to changes in the systemic microcirculation.

Our findings may not be generalizable to the adult population as a whole. Subsequent research should therefore aim at confirming the observations in larger and more diverse populations. In addition, it would be informative to study populations that may be more susceptible to microvascular effects of air pollutants due to underlying pathologies that promote chronic inflammation. Diabetics, for example, have been shown to be a vulnerable group for the effects of air pollution (von Klot et al. 2005; Jacobs et al., 2010).

We cannot exclude some exposure misclassification. Measurements from a monitoring station close to the study site were used to estimate exposures. However, participants may have been exposed to different BC concentrations at their place of residence or while commuting (Dons et al. 2012, Dons et al. 2013). The amount of time spent driving in traffic, as determined from the questionnaire, was negatively associated with arteriolar diameter, though the association was not statistically significant. Ideally, personal measurements of BC should be utilized in future studies.

The key finding of our repeated measurements study in a panel of healthy adults was that an acute narrowing of retinal arterial vessels, a marker for arteriolar damage, was associated with particulate matter air pollution. Based on our analysis, the estimated effect on CRAE, associated with a 10-$\mu g/m^3$ increase in average PM_{10} during the 24 hours before the retinal examination was equivalent to the change in CRAE associated with a 1.5-year increase in age. This microvascular response to air pollution might contribute to the development or progression of cardiovascular diseases and complications, as seen in epidemiological studies. Our findings add new evidence to the cardiovascular health effects of short-term exposure to air pollution in healthy people and suggest a mechanistic pathway through which air pollution can act as a trigger of cardiovascular events at least in part through effects on the microvasculature.

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Table 1. Descriptive population characteristics.

Personal Characteristics (n=84)	Mean ± SD or Number (%)
Age (y)	37 ± 9
Female (%)	44 (52%)
Race/ethnicity	
Caucasian (%)	83 (99%)
Asian (%)	1 (1%)
Smoking status	
Current	3 (4%)
General health characteristics	
Body Mass Index (kg/m²)	23 ± 3
Systolic Blood Pressure[a] (mm Hg)	126 ± 11
Diastolic Blood Pressure[a] (mm Hg)	75 ± 8
Heart rate[a] (bpm)	72 ± 13
Participation in traffic on day of examination	
Persons using a car	74 (88%)
Persons using a car in congested traffic	24 (30%)
Persons riding a bike or walking in traffic	27 (32%)

[a] Data were available for 59 subjects and reported blood pressure values are based on the average of three consecutive readings at two examination moments.

Table 2. Estimated change in mean Central Retinal Arteriolar Equivalent (CRAE) (95% confidence interval) in association with a 10-µg/m³ increase in PM10 or a 1-µg/m³ increase in BC. Both models include 84 persons; 25 had one measurement, 14 had 2 measurements and 45 had 3 measurements.

Exposure Time (lags)	**Model 1**[a]	**Model 2**[b]
PM_{10} (for each 10 µg/m³ increase)		
2 hours	-0.62 (-1.13, -0.11)*	-0.38 (-0.85, 0.08)
4 hours	-0.67 (-1.22, -0.13)*	-0.41 (-0.90, 0.09)
6 hours	-0.75 (-1.31, -0.18)*	-0.43 (-0.94, 0.09)
24 hours	-0.93 (-1.42, -0.45)***	-0.57 (-1.01, -0.12)*
2 days	-0.60 (-1.18, -0.02)*	-0.15 (-0.70, 0.40)
BC (for each 1 µg/m³ increase)		
2 hours	0.24 (-0.57, 1.05)	-0.03 (-0.75, 0.69)
4 hours	0.38 (-0.49, 1.26)	0.03 (-0.75, 0.82)
6 hours	0.52 (-0.47, 1.51)	0.10 (-0.79, 0.99)
24 hours	-1.84 (-3.18, -0.51)**	-1.54 (-2.69, -0.39)*
2 days	-0.21 (-1.13, 0.71)	-0.16 (-1.00, 0.68)

[a] Model 1 estimates were adjusted for: gender, age, BMI, smoking habits, alcohol and coffee consumption 24 hours prior to examination, time of the day and day of the week, outdoor temperature and barometric pressure.

[b] Model 2 includes model 1 covariates plus Central Retinal Venular Equivalent (CRVE).

Statistical differences are expressed as: *<0.05, ** <0.01, *** <0.001.

Table 3. Estimated change in Central Retinal Venular Equivalent (CRVE) in association with particulate air pollution (PM_{10}) and black carbon (BC). Estimates express the change (95% Confidence Intervals) in the retinal venular blood vessels associated with a 10-µg/m³ increase in PM_{10} or a 1-µg/m³ increase in BC. Both models include 84 persons; 25 had one measurement, 14 had 2 measurements and 45 had 3 measurements.

Exposure Time (lags)	**Model 1**[a]	**Model 2**[b]
PM_{10} (for each 10 µg/m³ increase)		
2 hours	-0.62 (-1.28, 0.04)	-0.39 (-1.00, 0.22)
4 hours	-0.77 (-1.48, -0.05)*	-0.49 (-1.15, 0.17)
6 hours	-0.93 (-1.67, -0.17)*	-0.60 (-1.28, 0.09)
24 hours	-0.86 (-1.42, -0.30)**	-0.60 (-1.26, 0.07)
2 days	-1.03 (-1.88, -0.18)*	-0.84 (-1.61, -0.08)*
BC (for each 1 µg/m³ increase)		
2 hours	0.46 (-0.65, 1.57)	0.29 (-0.71, 1.31)
4 hours	0.52 (-0.68, 1.73)	0.30 (-0.80, 1.40)
6 hours	0.47 (-0.87, 1.80)	0.22 (-1.01, 1.44)
24 hours	-1.18 (-3.11, 0.75)	-0.04 (-1.77, 1.70)

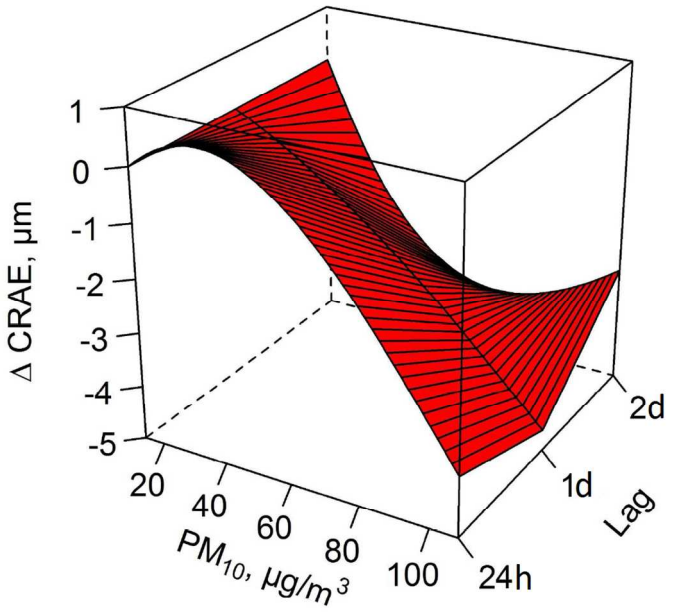
[a] **Model 1** estimates were adjusted for: gender, age, BMI, smoking habits, alcohol and coffee consumption 24 hours prior to examination, time of the day and day of the week, outdoor temperature and barometric pressure.

[b] **Model 2** also includes, in addition to covariates in model 1, Central Retinal Arteriolar Equivalent (CRAE).

Statistical differences are expressed as: * <0.05, ** <0.01, *** <0.001.

Figure Legend

Figure 1. Microvascular responses in association with short-term changes in air pollution. Unadjusted analysis for change in Central Retinal Arteriolar Equivalent (CRAE) in association with PM_{10}. The effect was estimated using restricted cubic splines with 5 knots located at the 5^{th}, 25^{th}, 50^{th}, 75^{th} and 95^{th} percentile for exposures on day of the examination using average exposure 24 hours before the clinical measurements (lag 24 h), and on the 24-hour average of the day before (lag 1d) and 48-hour average of the two preceding days (lag 2d).



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